(2)

DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188
Exp Date Jun 30, 1986

UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	3. DISTRIBUTION / AVAILABILITY OF REPORT This document has been approved for public release and sale; its distribution is unlimited.
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION WRAIR, Dept of Cellular Immunology	6b. OFFICE SYMBOL (*If applicable*) SGRD-UWF-C
7a. NAME OF MONITORING ORGANIZATION Walter Reed Army Institute of Research	
6c. ADDRESS (*City, State, and ZIP Code*) Walter Reed Army Institute of Research Dept of Cellular Immunology, Washington, D.C. 20307-5100	7b. ADDRESS (*City, State, and ZIP Code*) Library/Reprint Department Washington, D.C. 20307-5100
8a. NAME OF FUNDING / SPONSORING ORGANIZATION USAMRDC Ft Detrick, Frederick, MD 21701-5000	8b. OFFICE SYMBOL (*If applicable*)
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c. ADDRESS (*City, State, and ZIP Code*) Finance & Accounting Officer Ft Detrick, Frederick, MD 21701-5000	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO

11. TITLE (*Include Security Classification*)

Relative Inefficiency of Soluble Recombinant DC4 for Inhibition of Infection by Monocyte-Tropic HIV in Monocytes and T Cells

12. PERSONAL AUTHOR(S)

P. Gomatos, N. Stamatos, H. Gendelman, A. Fowler, D. Hoover, D. Kalter, D. Burke, E. Tramont & M. Meltzer

13a. TYPE OF REPORT	13b. TIME COVERED FROM _____ TO _____	14. DATE OF REPORT (*Year, Month, Day*)	15. PAGE COUNT

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (*Continue on reverse if necessary and identify by block number*)
FIELD	GROUP	SUB-GROUP	

19. ABSTRACT (*Continue on reverse if necessary and identify by block number*)

Macrophages are major viral reservoirs in the brain, lungs, and lymph nodes of HIV-infected patients. But not all HIV isolates infect macrophages. The molecular basis for this restrictive target cell tropism and the mechanisms by which HIV infects macrophages are not well understood: virus uptake by CD4-dependent and -independent pathways have both been proposed. Soluble rCD4 (sCD4) binds with high affinity to gp120, the envelope glycoprotein of HIV, and at relatively low concentrations (<1mg/ml) completely inhibits infection of many HIV strains in T cells or T cell lines. HTLV-IIIB infection of the H9 T cell line was completely inhibited by prior treatment of virus with 10mg/ml sCD4: no p24 Ag or HIV-induced t Cell syncytia were detected in cultures of H9 cells exposed to 1 x 10^4 $TCID_{50}$ HTLV-IIIB in the presence of sCD4. Under identical conditions and at a 100-fold lower viral inoculum, 10 mg/ml sCD4 had little or no effect on infection of monocytes by an of six different HIV isolates by three different criteria: p24 Ag release, virus-induced cytopathic effects, and the frequency of infected cells that express HIV-specific mRNA. At 10-to 100-fold higher concentrations of sCD4, however, infection was completely inhibited. Monoclonal anti-CD4 for inhibition of HIV infection in monocytes was a property of the virion, not the target cell: HIV isolates that infect both monocytes and T cells required similarly high levels of sCD4 (100 to 200 mg/ml) for inhibition of infection. These data suggest that the gp120 of progeny HIV derived from macrophages interacts with sCD4 differently than that of virions derived from T cells. For both variants of HIV, however, the predominant mechanism of virus entry for infection is DC4-dependent.

20 DISTRIBUTION / AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21 ABSTRACT SECURITY CLASSIFICATION
22a NAME OF RESPONSIBLE INDIVIDUAL	22b TELEPHONE (*Include Area Code*) / 22c. OFFICE SYMBOL

0022-1767/90/14411-4183$02.00/0
THE JOURNAL OF IMMUNOLOGY
Vol. 144, 4183-4188, No. 11, June 1, 1990
Printed in U.S.A.

RELATIVE INEFFICIENCY OF SOLUBLE RECOMBINANT CD4 FOR INHIBITION OF INFECTION BY MONOCYTE-TROPIC HIV IN MONOCYTES AND T CELLS[1]

PETER J. GOMATOS,* NICHOLAS M. STAMATOS,* HOWARD E. GENDELMAN,†‡ ARNOLD FOWLER,§ DAVID L. HOOVER,† D. CHESTER KALTER,†‡ DONALD S. BURKE,* EDMUND C. TRAMONT,* AND MONTE S. MELTZER[2]†

*From the †HIV Immunopathogenesis Program, Department of Cellular Immunology and *Division of Retrovirology, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100; §SRA Technologies Inc., and ‡Henry M. Jackson Foundation for the Advancement of Military Medicine, Rockville, MD 20850*

Macrophages are major viral reservoirs in the brain, lungs, and lymph nodes of HIV-infected patients. But not all HIV isolates infect macrophages. The molecular basis for this restrictive target cell tropism and the mechanisms by which HIV infects macrophages are not well understood: virus uptake by CD4-dependent and -independent pathways have both been proposed. Soluble rCD4 (sCD4) binds with high affinity to gp120, the envelope glycoprotein of HIV, and at relatively low concentrations (<1 μg/ml) completely inhibits infection of many HIV strains in T cells or T cell lines. HTLV-IIIB infection of the H9 T cell line was completely inhibited by prior treatment of virus with 10 μg/ml sCD4: no p24 Ag or HIV-induced T cell syncytia were detected in cultures of H9 cells exposed to 1×10^4 $TCID_{50}$ HTLV-IIIB in the presence of sCD4. Under identical conditions and at a 100-fold lower viral inoculum, 10 μg/ml sCD4 had little or no effect on infection of monocytes by any of six different HIV isolates by three different criteria: p24 Ag release, virus-induced cytopathic effects, and the frequency of infected cells that express HIV-specific mRNA. At 10- to 100-fold higher concentrations of sCD4, however, infection was completely inhibited. Monoclonal anti-CD4 also prevented infection of these same viral isolates in monocytes. The relative inefficiency of sCD4 for inhibition of HIV infection in monocytes was a property of the virion, not the target cell: HIV isolates that infect both monocytes and T cells required similarly high levels of sCD4 (100 to 200 μg/ml) for inhibition of infection. These data suggest that the gp120 of progeny HIV derived from macrophages interacts with sCD4 differently than that of virions derived from T cells. For both variants of HIV, however, the predominant mechanism of virus entry for infection is CD4-dependent.

Interaction between the HIV envelope glycoprotein, gp120, and T cell plasma membrane CD4 is a necessary event for infection: antibodies to CD4 inhibit HIV infection of T cells (1-3); certain human cells not normally susceptible to HIV develop a productive infection after transfection with CD4 cDNA (4). sCD4[3] also binds with high affinity to gp120 ($K_d \sim 10^{-9}$ M) and at relatively low concentrations (1 μg/ml) inhibits the infection of many HIV strains in T cells or T cell lines (5-10). But T cells are not the only target for HIV in infected patients. Macrophages of brain, lymph nodes, and lung are major virus reservoirs (11-13). The mechanisms by which HIV infects macrophages are not well understood. Indeed, laboratory strains of HIV-1 passaged in T cells or T cell lines (such as HTLV-IIIB) and used for many of the preceding studies do not readily infect monocytes (14-17). In contrast, virus recovered from blood leukocytes of HIV-infected patients onto monocyte target cells from uninfected donors can be readily passaged in other monocytes. For most seropositive patients, HIV isolates can be recovered from blood leukocytes in both the conventional T cell and monocyte cultures. Serial passage of these HIV clinical isolates into T lymphoblast and monocyte target cells documents a strong tropism of certain HIV for monocytes. HIV isolated in either T cells or monocytes can be serially passaged into cultures of PHA/IL-2-treated lymphoblasts. Analysis of such HIV-infected lymphoblasts by levels of p24 Ag and RT activity, in situ hybridization for HIV-specific mRNA, formation of cell syncytia during infection, down-modulation of T cell plasma membrane CD4, and transmission-electron microscopy (progeny virions budding at the plasma membrane only with no intracytoplasmic accumulation of viral particles) shows no qualitative or quantitative differences between T cell and monocyte-derived HIV isolates: these monocyte-tropic HIV grow equally well in monocytes and T cells (18). In marked contrast to the preceding results, viral isolates initially recovered from PHA/IL-2-treated lymphoblasts (T cell-tropic HIV) show little or no growth on monocytes by the criteria of virus-induced cytopathic effects, p24 Ag or RT activity levels, or infectious titer. Thus, HIV isolated in monocytes show dual tropism and infect monocytes and T cells equally; viruses isolated in PHA/IL-2-treated lymphoblasts replicate efficiently only in T cells (15-20).

Received for publication November 15, 1989.
Accepted for publication March 15, 1990.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] These studies were supported in part by the Henry M. Jackson Foundation for the Advancement of Military Medicine, Rockville, MD. H. [illegible] Carter-Wallace fellow of The Johns Hopkins University School of Public Health and Hygiene in the Department of Immunology and Infectious Diseases.
[2] Address correspondence and reprint requests to Monte S. Meltzer, Department of Cellular Immunology, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

[3] Abbreviations used in this paper: sCD4, soluble rCD4; RT, reverse transcriptase; MCSF, macrophage-CSF; $TCID_{50}$ 50% tissue culture infective dose.

We show in this report that pretreatment of monocytes with mAb against CD4 completely prevented productive infection by monocyte-tropic HIV. However, under conditions in which sCD4 completely inhibited HTLV-IIIB infection of T cells, this recombinant molecule had little or no effect on the ability of six different monocyte-tropic HIV strains to infect macrophages. The apparent refractoriness of these monocyte-tropic HIV to inhibition by sCD4 was quantitative and evident with both monocyte and T cell targets. Under identical conditions and taking into account the amount of sCD4 used and the viral inoculum, there was at least a 10,000-fold difference in the efficacy of sCD4 for inhibition of monocyte-tropic HIV infection in monocytes vs that of HTLV-IIIB in T cells.

MATERIALS AND METHODS

Isolation and culture of monocyte, T lymphoblast, or H9 target cells. Monocytes were recovered from PBMC of HIV and hepatitis B-seronegative donors after leukapheresis and purified by countercurrent centrifugal elutriation of mononuclear leukocyte-rich fractions of blood cells. Cell suspensions were >98% monocytes by criteria of cell morphology on Wrightstained cytosmears, by granular peroxidase, and by nonspecific esterase. Monocytes were cultured as adherent cell monolayers (1.5×10^5 cells/6-mm tissue culture well) in 0.15 ml DMEM (formula 78-176AJ, GIBCO, Grand Island, NY) supplemented with 10% heat-inactivated A^+ human serum, 50 μg/ml gentamicin, and 1000 U/ml recombinant human MCSF (a generous gift from the Cetus Corporation, Emeryville, CA) (17). The H9 T cell lymphoma cell line (Dr. R. Gallo, contributor, AIDS Research and Reference Reagent Program, AIDS Program, National Institute of Allergy and Infectious Diseases, Bethesda, MD) was cultured in RPMI 1640 medium (GIBCO) with gentamicin, 15% FCS (Sterile Systems, Inc., Logan, UT), and 10% partially purified human IL-2 (Advanced Biotechnologies, Inc., Columbia, MD). PBMC, isolated from whole blood by Ficoll-diatrizoate density gradient centrifugation, were cultured at 1×10^6 viable cells/ml in RPMI 1640 medium (GIBCO) with 5 μg/ml PHA (Sigma Chemical Co., St. Louis, MO), 10% purified human IL-2 (Advanced Biotechnologies), and 15% heat-inactivated FCS (Sterile Systems).

HIV infection of monocyte and T cell targets. HTLV-IIIB/H9 (Dr. R. Gallo, contributor, AIDS Research and Reference Reagent Program, AIDS Program, NIAID, NIH), an HIV strain adapted to T cell lines, was pretreated with dilutions of sCD4 (a generous gift of Biogen, Cambridge, MA) for 30 min at 37° C and added to H9 cells or PBMC-derived lymphoblasts treated 3 days previously with PHA/IL-2. For virus infection in PHA/IL-2-treated lymphoblasts, all cultures were supplemented with 2 μg/ml Polybrene® (Sigma). MCSF-treated monocytes were cultured as adherent monolayers 5 to 10 days before use as target cells. Monocytes were exposed to any of several HIV originally isolated and passaged in MCSF-treated monocytes (strains ADA, 16, 24, 36, 37, and 38) (17, 18). Monocyte-tropic HIV were also pretreated with dilutions of sCD4 for 30 min at 37° C. Except where noted, dilutions of sCD4 were maintained with both H9 and monocytes throughout the culture interval. All cultures were refed with fresh medium every 2 to 3 days. Levels of p24 Ag in culture fluids were determined by ELISA (Coulter Electronics Inc., Hialeah, FL).

In situ hybridization with HIV RNA probes. Single-stranded HIV ^{35}S-RNA probes were synthesized from rDNA plasmid containing SP6/T7 promoters (Promega Biotec, Madison, WI) and incubated in alkaline (pH 10.2) solutions before hybridization to facilitate entry into cells. Cytosmears of monocytes on polylysine-coated glass slides were fixed in periodate-lysine paraformaldehyde-glutaraldehyde and pretreated with proteinase K, triethanolamine and HCl. Specimens were prehybridized in 10 mM Tris, pH 7.4, 2 × SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.4), Denhardt's solution (0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% BSA), and 200 μg/ml yeast tRNA at 45° C for 2 h and hybridized in this solution with 10% dextran sulfate, 5 μM dithiothreitol, and 1×10^6 cpm ^{35}S-labeled HIV RNA. Slides were serially washed in solutions with RNase to reduce binding of nonhybridized probe. Autoradiography was performed in absolute darkness (11).

RESULTS AND DISCUSSION

Initial studies confirmed previous reports that HTLV-IIIB infection of the H9 T cell line was completely inhibited by prior treatment of virus with 10 μg/ml sCD4 and with the same concentration of sCD4 continuously maintained in culture (Fig. 1). No p24 Ag was detected by ELISA in culture fluids of H9 cells exposed to 1×10^4 $TCID_{50}$ HTLV-IIIB in the presence of sCD4 (or in replicate experiments with 1×10^5 $TCID_{50}$ HTLV-IIIB). Whereas HTLV-IIIB-infected H9 cells showed about 215 syncytia/1×10^5 cells in triplicate cultures at 7 days, no syncytia were observed in replicate cultures treated with 10 μg/ml sCD4. In other experiments and as previously reported, HTLV-IIIB infection of H9 cells was completely inhibited by as little as 0.1 to 1 μg/ml sCD4 (5–10). Inhibition of infection by sCD4 was also evident with HTLV-IIIB and PHA/IL-2-treated lymphoblasts from PBMC: no p24 Ag was detected through 3 wk in culture fluids of PHA/IL-2-treated lymphoblasts exposed to 1×10^4 $TCID_{50}$ HTLV-IIIB in the presence of 10 μg/ml sCD4; no HIV-induced syncytia were evident among the cells of these same sCD4-treated cultures. Similar results have been reported by others with at least five different HIV strains in both T cells of peripheral blood and T cell lines (5–10).

Under identical conditions, sCD4 at 10 μg/ml had little or no effect on levels of p24 Ag in culture fluids of monocytes infected with 1×10^2 $TCID_{50}$ ADA, a monocyte-tropic HIV patient isolate (Fig. 1). It is important to note that the HTLV-IIIB used for infection of H9 cells in these experiments contained >100-fold more infectious particles than the ADA inoculum used to infect monocytes. Thus, under identical conditions and taking into account both the amount of sCD4 used and the viral inoculum, there was at least a 10,000-fold difference in the efficacy of sCD4 for inhibition of HIV infection in T cells vs macrophages.

The inability of sCD4 to inhibit HIV infection of monocytes was confirmed by two other independent means of detecting virus infection. HIV induces a characteristic cytopathic effect in monocyte cultures: formation of multinucleated giant cells (16, 17, 21). Figure 2 shows

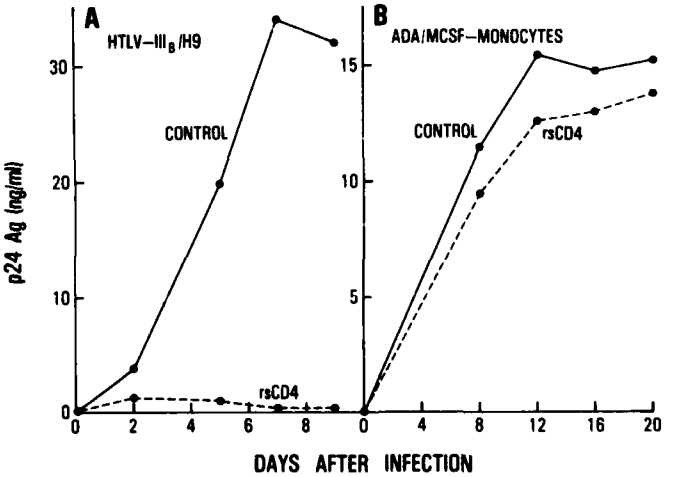

Figure 1. Effect of sCD4 on HIV infection of H9 T cells and MCSF-treated monocytes. The H9 T cell lymphoma cell line was cultured in RPMI 1640 medium with gentamicin, 15% FCS, 2 μg/ml Polybrene®, and 10% partially purified human IL-2 at 1×10^5 cells/6-mm culture well. HTLV-IIIB was pretreated with 10 μg/ml sCD4 for 30 min at 37° C and added at 1×10^4 $TCID_{50}$ to the H9 cells. PBMC purified to >98% monocytes were incubated as adherent monolayers at 1.5×10^5 cells/6-mm culture well in 0.15 ml DMEM with 10% human serum, and 1000 U/ml MCSF. At 7 to 10 days, monocytes were exposed to 1×10^2 $TCID_{50}$ ADA, a monocyte-tropic HIV isolate pretreated with 10 μg/ml sCD4. The sCD4 was maintained at 10 μg/ml with both H9 and monocytes throughout the culture interval. All cultures were refed with fresh medium every 2 to 3 days. Levels of p24 Ag in culture fluids were determined by ELISA.

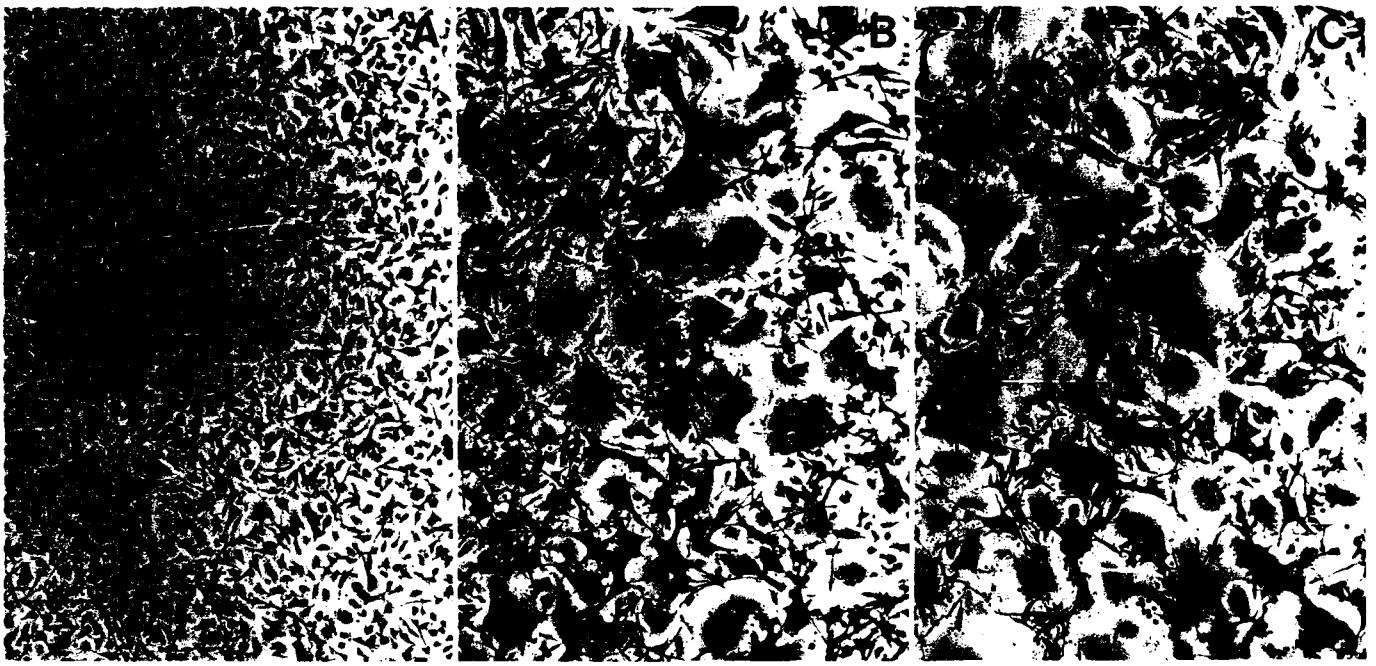

Figure 2. Effect of sCD4 on HIV-induced cytopathic effects in MCSF-treated monocytes. PBMC purified to > 98% monocytes were incubated as adherent monolayers in medium with 10% human serum and 1000 U/ml MCSF. At 7 to 10 days, monocytes were exposed to 1×10^2 $TCID_{50}$ ADA pretreated with 10 μg/ml sCD4. The sCD4 was maintained at 10 μg/ml throughout the culture interval. Cultures were refed with fresh medium every 2 to 3 days. Photomicrographs of adherent cells fixed in methanol and Wright-stained 15 days after infection are at 200× original magnification. *Left panel:* uninfected MCSF-treated monocytes; *middle panel:* HIV-infected monocytes; *right panel:* HIV-infected monocytes with 10 μg/ml sCD4.

control monocytes without multinucleated giant cells in culture for 3 wk. Multinucleated giant cells were evident in about 20 to 40% of total cells in the HIV-infected cultures. There was no difference in the number or extent of these multinucleated giant cells in replicate cultures treated with 10 μg/ml sCD4. In similar experiments, about 27 ± 2% of total monocytes (mean ± sem of 200 cells in duplicate determinations) expressed HIV mRNA as detected by in situ hybridization with radiolabeled ssRNA probes at 17 days. The frequency of monocytes that express HIV mRNA in cultures with 10 μg/ml sCD4 was 31 ± 2%.

The inability of sCD4 to inhibit infection of HIV in monocytes was not limited to the ADA isolate. We examined six different HIV isolates that infect monocytes (strains ADA, 16, 24, 36, 37, and 38). In each case, the HIV inoculum was less than 0.001 monocyte infectious doses per cell. Results for four of these isolates (strains 16, 24, 36, and 38) are shown in Figure 3. In each instance, 10 μg/ml sCD4 had little or no effect on virus infection: levels of p24 Ag released into monocyte culture fluids at 3 wk with or without sCD4 were indistinguishable. In separate experiments, we obtained identical results when we used as an inoculum viral stock that had been pelleted after ultracentrifugation. Such washing after centrifugation would remove any soluble gp120 present in the virus stock that could potentially have interacted with the sCD4.

The preceding results showed that 10 μg/ml sCD4 had little or no effect on infection of monocytes by any of six different HIV isolates by three different criteria: p24 Ag release, virus-induced cytopathic effects, and the frequency of infected cells that express HIV-specific mRNA. The role of plasma membrane CD4 in the initiation of infection by HIV in monocytes was further examined by two different approaches by block these receptors: a)

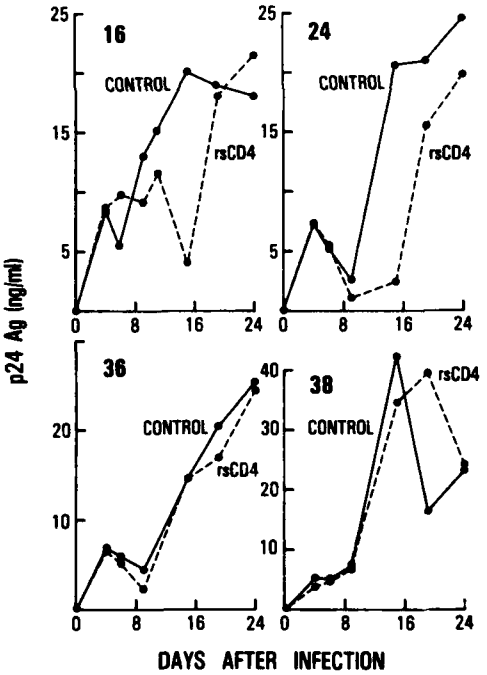

Figure 3. Effect of sCD4 on HIV infection of MCSF-treated monocytes. PBMC purified to >98% monocytes were incubated as adherent monolayers in medium with 10% human serum, and 1000 U/ml MCSF. At 7 to 10 days, monocytes were exposed to 1×10^2 $TCID_{50}$ HIV isolates 16, 24, 36 and 38 pretreated with 10 μg/ml sCD4. The sCD4 was maintained at 10 μg/ml throughout the culture interval. All cultures were refed with fresh medium every 2 to 3 days. Levels of p24 Ag in culture fluids were determined by ELISA.

competitive inhibition by excess HTLV-IIIB, an HIV strain that interacts with CD4 receptors in many different cell lines but fails to infect monocytes, and b) use of mAb that bind to epitopes on CD4 close to or at the site of binding for virus gp120. HTLV-IIIB does not grow in monocytes, but it does enter these cells and is present in

endocytic vacuoles shortly after adsorption (14). We exposed monocytes to HTLV-IIIB at an inoculum titer 1000- to 10,000-fold more than that used for any of the monocyte-tropic HIV isolates (Table I). The time course and ultimate levels of p24 Ag released into monocyte culture fluids after infection with any of the monocyte-tropic HIV isolates were unchanged by prior exposure of these cells to an excess of HTLV-IIIB. No p24 Ag was detected in culture fluids of monocytes exposed to HTLV-IIIB alone through 3 wk. Thus, if HTLV-IIIB occupied the CD4 receptors on the monocytes, such receptor occupancy did not affect the ability of monocyte-tropic HIV isolates to initiate a productive infection.

These observations raise the possibility of another receptor or mechanism of entry for HIV in monocytes independent of CD4. However, this possibility was made less likely by subsequent experiments with mAb directed against the gp120 binding site on CD4. Infection of monocytes by monocyte-tropic HIV isolates was completely abrogated by prior treatment of cells with the mAb, Leu 3a (Table I) or OKT4a (data not shown). As a control, equal amounts of mAb directed against HLA-DR had no effect.

These data document an apparent paradox. mAb against CD4 completely inhibited infection of any of three different monocyte-tropic HIV isolates in monocytes. This observation strongly suggests that the interaction between HIV and monocyte CD4 is a necessary event for the initiation of infection. Yet, sCD4 at levels 1000- to 10,000-fold higher than those required to inhibit HTLV-IIIB infection of T cells, fails to inhibit the infection of monocyte-tropic HIV isolates in monocytes. If CD4 is the receptor for HIV on monocytes, then the interaction of sCD4 with monocyte-tropic HIV may differ from that of T cell-tropic HIV (perhaps similar in concept to previously observed differences in efficacy of sCD4 for inhibition of HIV-1 and HIV-2 infection) (10). Furthermore, the interaction of sCD4 which lacks the transmembrane and cytoplasmic sequences of CD4 (6–9) with monocyte-tropic HIV may be different from that of the virus with CD4 on monocyte plasma membranes. Such differences in the interaction of monocyte-tropic and T cell-tropic HIV to sCD4 may be quantitative or qualitative.

To address these issues, we reexamined the effect of sCD4 on HIV infection of monocytes at concentrations 60-fold higher than that previously used. HTLV-IIIB (about 1×10^4 $TCID_{50}$) was pretreated with 5 to 625 μg/ml sCD4 for 1 h and then the mixture was added to H9 target cells. The H9 cells were exposed to HIV in the presence of sCD4 for 24 h. All cultures were refed by removing half of the volume and replacing this with medium without sCD4 every 2 days. At 10 days, p24 Ag levels in cultures of H9 cells exposed to HTLV-IIIB without sCD4 exceeded 50 ng/ml. No p24 Ag was detected (≤0.05 ng/ml) in any culture treated with sCD4. The effect of sCD4 on the capacity of monocyte-tropic HIV isolates ADA and 36 (about 50 $TCID_{50}$) to infect monocytes was determined under identical conditions. In these experiments, sCD4 completely inhibited the infection of monocyte-tropic HIV in monocytes (Table II). Little or no p24 Ag was detected in monocyte cultures exposed to HIV isolate 36 pretreated with 625 μg/ml sCD4 or to HIV isolate ADA pretreated with 125 μg/ml sCD4 at any time during 3 wk. Similarly, levels of RT activity in these culture fluids were also undetectable. RT activity in the infected monocyte control cultures were 4×10^5 cpm/ml at 10 days (background RT activity levels of $<5 \times 10^3$ cpm/ml). The concentration of sCD4 required to inhibit 50% maximum levels of p24 Ag in the HIV-infected monocyte cultures was 250 μg/ml for HIV isolate 36 and 45 μg/ml for HIV isolate ADA. Thus sCD4 completely inhibited HIV infection of monocytes, but the relative efficiency of this inhibition compared to that for T cells differed by more than 10,000-fold.

The progeny virus of HIV-infected monocytes show marked differences from those of HIV-infected T cells. Most notable in these differences is the relative proportion of *gag* vs *env* gene products. The *env* gene products in the progeny virus of HIV-infected T cells represent about 35% of total viral protein (gp160 + gp120/gp160 + gp120 + p55 + p24) by radioimmunoprecipitation analysis of virions with sera from HIV-infected patients. In contrast, the *env* gene products in progeny virus of HIV-infected monocytes represent <10% of total virus protein (22). Transmission electron microscopic analysis of progeny virus from infected monocytes and T cells confirm the radioimmunoprecipitation analysis. Characteristic envelope "spikes", the morphologic representation of gp120, are evident in the HIV released from infected T cells. The progeny virus of HIV-infected monocytes show little or no "spikes" and are relatively bald (21). These observations document a substantial quantitative decrease in the amount of envelope in progeny virus released from infected monocytes and may explain differences in the interaction of sCD4 with the gp120 of monocyte and T cell-tropic HIV.

Monocyte-tropic HIV isolates preferentially infect monocytes, but will also infect T cells (18). With certain

TABLE I

Effect of monoclonal anti-CD4 or HTLV-IIIB pretreatment on HIV infection of monocytes[a]

HIV Isolate	Release of p24 Ag (ng/ml) in cultures treated with:					
	Leu 3a anti-CD4				anti-HLA DR	HTLV-IIIB
	1/30	1/150	1/750	∞	1/30	
ADA	0	1	4	5	>6	>4
16	0	1	7	8	>6	>4
36	0	3	11	11	>6	>4

[a] PBMC purified to > 98% monocytes were incubated as adherent monolayers in medium with 10% human serum, and 1000 U/ml MCSF. At 7 to 10 days, monocytes were treated with dilutions of Leu 3a anti-CD4 or anti-HLA DR (Becton Dickinson Immunocytochemistry Systems, Mountain View, CA) or 1×10^4 $TCID_{50}$ HTLV-IIIB. After 30-min treatment, monocytes were exposed to 1×10^1 $TCID_{50}$ of three different monocyte-tropic HIV isolates. After virus adsorption, cultures were washed and refed with fresh medium every 2 to 3 days through 3 wk. Monocytes were continuously exposed to dilutions of mAb throughout the 3-wk interval. Levels of p24 Ag in culture fluids were determined by ELISA. The p24 Ag in monocyte cultures treated with HTLV-IIIB alone was < 0.05 ng/ml through 3 wk. Results in Table I represent one of four replicate experiments.

TABLE II

Effect of sCD4 on ability of monocyte-tropic HIV to infect MCSF-treated monocytes or PHA/IL-2-treated lymphoblasts[a]

sCD4 (μg/ml)	Release of p24 Ag (ng/ml) in cultures infected with:			
	HIV Isolate 36		HIV Isolate ADA	
	Monocytes	Lymphoblasts	Monocytes	Lymphoblasts
0	>6 (390)	104	>6 (366)	71
5	>6 (370)	106	>6 (288)	80
25	>6 (238)	72	6 (144)	66
125	6 (122)	55	1 (30)	51
625	0 (4)	7	0 (6)	1

[a] Unfractionated PBMC were cultured for 3 days in RPMI 1640 medium with 15% FCS, 2 μg/ml Polybrene*, and 10% human IL-2 at 1×10^5 cells/6-mm culture well. PBMC purified to > 98% monocytes were incubated 7 days as adherent monolayers at 1.5×10^5 cells/6-mm culture wells in medium with 10% human serum, and 1000 U/ml MCSF. HIV isolates ADA and 36 (1×10^3 $TCID_{50}$) were pretreated with different concentrations of sCD4 for 1 h at 37°C, and the mixture added to monocytes and lymphoblasts. Target cells were exposed to HIV in the presence of sCD4 for 24 h. All cultures were refed by removing half of the medium and replacing this with medium without sCD4 every 2 days. Levels of p24 Ag in culture fluids were determined by ELISA. Data shown are p24 Ag levels 3 wk after HIV infection. Levels of RT activity (cpm/ml $\times 10^{-3}$) in culture fluids are shown in parentheses. RT activity in culture fluids of uninfected cells was < 5000 cpm/ml. Results represent one of three replicate experiments.

monocyte-tropic HIV isolates this amphotropism can be truly equal. In contrast, HIV strains initially isolated in T cells and passaged only in T cell targets do not infect monocytes. If the relative inefficiency of sCD4 for inhibition of HIV infection of monocytes is a property of the virus (and not the target cell), then this quality might also be expressed with T cell targets. Monocyte-tropic HIV isolates 36 and ADA also replicate in PHA/IL-2-treated lymphoblasts from PBMC. In contrast to previous observations with the HTLV-IIIB strain, sCD4 at 10 μg/ml failed to inhibit infection of either monocyte-tropic HIV isolate in T cells. Higher concentrations of sCD4, however, completely prevented infection. No p24 Ag was detected through 2 wk of culture with PHA/IL-2-treated lymphoblasts exposed to monocyte-tropic HIV isolates 36 or ADA pretreated with 625 μg/ml sCD4 (Table II). The concentration of sCD4 required to inhibit 50% maximum levels of p24 Ag in the HIV-infected PHA/IL-2-treated lymphoblast cultures was 180 μg/ml for HIV isolate 36 and 130 μg/ml for HIV isolate ADA. These data strongly suggest that the relative inefficiency of sCD4 for inhibition of monocyte-tropic HIV infection of monocytes or T cells is a property of the virion and not of the target cell.

The preceding data is consistent with the hypothesis that HIV interaction with CD4 is an obligate reaction for infection of both T cells and monocytes. None of these observations, however, preclude another, CD4-independent route of infection. The frequency of monocytes that express plasma membrane CD4 may range from 5 to 90% (15, 17, 23–26). Expression of CD4 on infected macrophages in the central nervous system, the predominant target cell for HIV in this tissue, may be especially low (27). HIV may enter macrophages through phagocytosis (14), FcR-mediated endocytosis (28), or interaction with receptors for mannosylated proteins (29). Several recent reports document at least one CD4-independent route of infection in susceptible T cell and monocyte targets (19, 28, 30, 31). Infection of T cells or monocytes with HIV was markedly enhanced (5- to 10-fold increase in RT activity in culture fluids) by sera from certain HIV-infected patients. Antibody-mediated enhancement in both monocytes and T cells was not inhibited by monoclonal anti-CD4 (Leu 3a) (19). In monocytes, antibody-mediated enhancement of HIV infection is inhibited by monoclonal anti-FcRIII (the predominant Fc receptor in tissue macrophages and monocytes in culture, but absent on circulating blood monocytes) but not mAb directed against FcRI or FcRII (19). Antibody-mediated enhancement of HIV infection in the myeloid cell line U937, which lacks FcRIII but does express FcRI and FcRII, was blocked by heat-aggregated IgG (28). In these studies, the infection pattern of U937 simulates more closely that of T cells rather than that of monocytes or macrophages. U937 is also a susceptible target cell for several T cell-tropic HIV, but not monocyte-tropic viruses (20). Interestingly, infection of U937 by HTLVIII-B or other T cell-tropic HIV is also blocked by relatively low concentrations of sCD4 (10).

By whatever mechanism, the preceding data convincingly shows that sCD4 is much less effective in blocking infection of HIV derived from infected monocytes than of virions derived from T cells. If our results obtained in cell culture systems have significance for patients, then macrophages that serve as reservoirs for HIV throughout the body and may ultimately be responsible for chronic central nervous system disease, may not be protected by sCD4 treatment.

Acknowledgments. We are indebted to John A. Wohlhieter for help and discussion. The authors also thank members of the Walter Reed Retroviral Research Group for excellent patient management.

REFERENCES

1. **Dalgleish, A. G., P. C. L. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature 312:763.*
2. **Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercend. J. C. Gluckman, and L. Montagnier.** 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature 312:767.*
3. **Sattentau, Q. J., A. G. Dalgleish, R. A. Weiss, and P. C. L. Beverley.** 1986. Epitopes of the CD4 antigen and HIV infection. *Science 234:1120.*
4. **Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell 47:333.*
5. **Smith, D. H., R. A. Byrn, S. A. Marsters, T. Gregory, J. E. Groopman, and D. J. Capon.** 1987. Blocking of HIV-1 infectivity by a soluble, secreted form of the CD4 antigen. *Science 238:1704.*
6. **Fisher, R. A., J. M. Bertonis, W. Meier, V. A. Jophnson, D. S. Costopoulos, T. Liu, R. Tizard, B. D. Walker, M. S. Hirsch, R. T. Schooley, and R. A. Flavell.** 1988. HIV infection is blocked in vitro by recombinant soluble CD4. *Nature 331:76.*
7. **Hussey, R. E., N. E. Richardson, M. Kowalski, N. R. Brown, H.-C. Chang, R. F. Siliciano, T. Dorfman, B. Walker, J. Sodroski, and E. L. Reinherz.** 1988. A soluble CD4 protein selectively inhibits HIV replication and syncytium formation. *Nature 331:78.*
8. **Deen, K. C., J. S. McDougal, R. Inacker, G. Folena-Wasserman, J. Arthos, J. Rosenberg, P. J. Maddon, R. Axel, and R. W. Sweet.** 1988. A soluble form of CD4 (T4) protein inhibits AIDS virus infection. *Nature 331:82.*
9. **Traunecker, A., W. Luke, and K. Karjalainen.** 1988. Soluble CD4 molecules neutralize human immunodeficiency virus type 1. *Nature 331:84.*
10. **Clapham, P. R., J. N. Weber, D. Whitby, K. McIntosh, A. G. Dalgleish, P. J. Maddon, K. C. Deen, R. W. Sweet, and R. A. Weiss.** 1989. Soluble CD4 blocks the infectivity of diverse strains of HIV and SIV for T cells and monocytes but not for brain and muscle cells. *Nature 337:368.*
11. **Koenig, S., H. E. Gendelman, J. M. Orenstein, M. C. Dal Canto, G. M. Pezeshkpour, M. Yungbluth, F. Janotta, A. Aksamit, M. A. Martin, and A. S. Fauci.** 1986. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science 233:1089.*

12. **Armstrong, J. A., and R. Horne.** 1984. Follicular dendritic cells and virus-like particles in AIDS-related lymphadenopathy. *Lancet II:370.*
13. **Tschachler, E., V. Groh, M. Popovic, D. L. Mann, K. Konrad, B. Safai, L. Eron, F. diMarzo-Veronese, K. Wolff, and G. Stingl.** 1987. Epidermal Langerhans cells-a target for HTLV-III/LAV infection. *J. Invest. Dermatol. 88:233.*
14. **Nicholson, J. K. A., G. D. Cross, C. S. Callaway, and J. S. McDougal.** 1986. In vitro infection of human monocytes with human T lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV). *J. Immunol. 137:323.*
15. **Gartner, S., P. Markovits, D. M. Markovitz, M. H. Kaplan, R. C. Gallo, and M. Popovic.** 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science 233:215.*
16. **Perno, C.-F., R. Yarchoan, D. A. Cooney, N. R. Hartman, D. S. Webb, A. Z. Hao, H. Mitsuya, D. G. Johns, and S. Broder.** 1989. Replication of human immunodeficiency virus in monocytes: granulocyte/ macrophage colony stimulating factor (GM-CSF) potentiates viral production yet enhances the antiviral effect mediated by 3'azido-2'3'-dideoxythymidine (AZT) and other dideoxynucleoside congeners of thymidine. *J. Exp. Med. 169:933.*
17. **Gendelman, H. E., J. M. Orenstein, M. A. Martin, C. Ferrua, R. T. Mitra, T. Phipps, L. A. Wahl, H. C. Lane, A. S. Fauci, D. S. Burke, D. Skillman, and M. S. Meltzer.** 1988. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony stimulating factor 1-treated monocytes. *J. Exp. Med. 167:1428.*
18. **Gendelman, H. E., L. Baca, H. Husayni, J. M. Orenstein, J. A. Turpin, D. Skillman, D. L. Hoover, and M. S. Meltzer.** 1990. Macrophage-human immunodeficiency virus interaction: viral isolation and target cell tropism. *AIDS 4:221.*
19. **Homsy, J., M. Meyer, M. Tateno, S. Clarkson, and J. A. Levy.** 1989. The Fc and not CD4 receptor mediates antibody enhancement of HIV infection in human cells. *Science 244:1357.*
20. **Koyanagi, Y., S. Miles, R. T. Mitsuyasu, J. E. Merrill, H. V. Vinters, and I. S. Y. Chen.** 1987. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropism. *Science 236:819.*
21. **Orenstein, J. M., M. S. Meltzer, T. Phipps, and H. E. Gendelman.** 1988. Cytoplasmic assembly and accumulation of human immunodeficiency virus types 1 and 2 in recombinant human colony stimulating factor-1 treated human monocytes: an ultrastructural study. *J. Virol. 62:2578.*
22. **Hansen, B. D., M. S. Meltzer, J. Hoxie, and H. E. Gendelman.** 1989. Studies of CD4 binding, biosynthesis, and processing of human immunodeficiency virus-infected monocytes and T cells. In *Abstracts, Modern Approaches to New Vaccines Including Prevention of AIDS.* Cold Spring Harbor Laboratory, Cold Spring, NY. p. 66.
23. **Haas, J. G., G. Riethmuller, and H. W. L. Ziegler-Heitbrock.** 1987. Monocyte phenotype and function in patients with the acquired immunodeficiency syndrome (AIDS) and AIDS-related disorders. *Scand. J. Immunol. 26:371.*
24. **Crowe, S., J. Mills, and M. S. McGrath.** 1987. Quantitative immunocytofluorographic analysis of CD4 antigen expression and HIV infection of human peripheral blood monocyte/macrophages. *AIDS Res. Hum. Retroviruses 3:135.*
25. **Faltynek, C. R., L. R. Finch, P. Miller, and W. R. Overton.** 1989. Treatment with recombinent IFNγ decreases cell surface CD4 levels on peripheral blood monocytes and on myelomonocytic cell lines. *J. Immunol. 142:500.*
26. **McElrath, M. J., J. E. Pruett, and Z. A. Cohn.** 1989. Mononuclear phagocytes of blood and bone marrow: comparative roles as viral reservoirs in human immunodeficiency virus type 1 infections. *Proc. Natl. Acad. Sci. USA 86:675.*
27. **Vazeux, R., N. Brousse, A. Jarry, D. Henin, C. Marche, C. Vedrenne, J. Mikol, M. Wolff, C. Michon, W. Rozenbaum, J.-F. Bureau, L. Montagnier, and M. Brahic.** 1987. AIDS subacute encephalitis. Identification of HIV-infected cells. *Am. J. Pathol. 126:403.*
28. **Takeda, A., C. U. Tuazon, and F. A. Ennis.** 1988. Antibody-enhanced infection by HIV-1 via Fc receptor mediated entry. *Science 242:580.*
29. **Ezekowitz, R. A. B., M. Kuhlman, J. E. Groopman, and R. A. Byrn.** 1989. A human serum mannose-binding protein inhibits in vitro infection by the human immunodeficiency virus. *J. Exp. Med. 169:185.*
30. **Robinson, W. E., Jr., D. C. Montefiori, and W. M. Mitchell.** 1988. Antibody-dependent enhancement of human immunodeficiency virus type-1 infection. *Lancet I:790.*
31. **Homsy, J., M. Tateno, and J. A. Levy.** 1988. Antibody-dependent enhancement of HIV infection. *Lancet I:1285.*